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Identification of eggplant (S*olanum melongena*) as a new host of begomovirus *Pepper yellow vein Mali virus* in Côte d'Ivoire

Kolotcholohofolo Soro[1*], Thérèse Atcham Agneroh[1], Kouakou Théodore Kouadio[1]
[1]Institut National Polytechnique Félix Houphouët-Boigny de Yamoussoukro, P.O. Box 1093, Yamoussoukro, Côte d'Ivoire, West Africa
Corresponding author: Email: phytiatresoro2011@gmail.com Tel: +225 49079639

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ABSTRACT
Objective: Eggplant (*Solanum melongena*) is one of the important vegetables in Africa and Asia. Begomoviruses are emerging plant viruses that cause significant losses. However, there is little research on begomoviruses infecting eggplant. Therefore, this study aimed at identifying begomoviruses infecting eggplant.
Methodology and results: Six samples of virus-like infected eggplants were collected in Ferkessedougou in the North of Cote d'Ivoire. The molecular tests *Polymerase Chain Reaction* (PCR) and *Rolling Circle Amplification* (RCA) were performed on the samples. One sample tested positive by PCR and RCA while the five others were negative by PCR for begomoviruses. Products from both tests were sequenced to get partial sequence of begomovirus *Pepper yellow vein Mali virus* (PepYVMLV) from PCR and two full genome components DNA A and DNA B of PepYVMLV from RCA. The sequences were released in Genbank.
Conclusion and application of findings: This study has done the molecular characterization of the complete two genome sequence components DNA A and DNA B of *Pepper yellow vein Mali* on eggplant. Agro-infection of eggplants with the two components could reveal actual specific symptoms which are caused by PepYVMLV on eggplant. This could help opens possibilities of engineering resistant eggplant to PepYMLV.
Keywords: Eggplant, begomovirus, *Pepper yellow vein Mali virus*, new host, Cote d'Ivoire

RESUME
Objectif: L'aubergine (*Solanum melongena*) est l'un des légumes les plus importants en Afrique et en Asie. Les begomovirus sont des virus émergents qui causent de pertes importantes. Toutefois, il y a très peu de recherches sur les begomovirus de l'aubergine. Ainsi, cette étude visait à l'identification des begomovirus infectant l'aubergine.
Méthodologie et résultats: Nous avons collecté 6 échantillons d'aubergine à Ferkéssédougou au Nord de la Côte d'Ivoire, parmi des plants d'aubergine qui présentaient des symptômes de type viral. Les tests moléculaires *Polymerase Chain Reaction* (PCR) et *Rolling Circle Amplification* (RCA) ont été réalisés sur les échantillons. Un échantillon a été positif à la fois à la PCR et la RCA alors que les 5 autres étaient négatifs à la PCR pour les begomovirus. Le séquençage des produits de la PCR a donné une séquence partielle du

begomovirus *Pepper yellow vein Mali virus* (PepYVMLV). Les produits issus de la RCA ont donné des séquences des composants ADN A et ADN B de PepYVMLV qui ont été publiées dans le Genbank.
Conclusion et application des résultats: Notre étude a effectué la caractérisation moléculaire des deux sequences complètes des composantes DNA A et DNA B du génome complet du *Pepper yellow vein Mali virus* sur l'aubergine. L'agro-infectioon des aubergines avec les deux composantes pourrait révéler les symptômes spécifiques réels qui sont causés par le PepYVMLV sur l'aubergine. Cela pourrait ouvrir des possibilités de mise en place de variétés d'aubergines résistantes au PepYMLV.
Mots-clés: Aubergine, begomovirus, *Pepper yellow vein Mali virus*, nouvelle plante hôte, Côte d'Ivoire

INTRODUCTION

Begomovirus genomes are DNA monopartite or bipartite (DNA A and DNA B) circular 2.5 to 3.64 Kb DNA encapsidated in twinned icosahedral particles (Navas-Castillo *et al.,* 2011; Zerbini *et al.,* 2017). The economic losses due to begomovirus infections are significant and represent billions of US dollars a year worldwide (Leke *et al.,* 2015). In India, begomoviruses cause yield losses, which worth about 300 million USD on legume crops (Varma *et al.,* 2003). On tomato crops, the begomovirus Tomato leaf curl viruses (ToLCVs) caused a loss of 140 million USD in Florida, USA (Moffat, 1999). Cassava mosaic viruses are well-known begomoviruses in Africa. Yield loses due to CMV are estimated to $1200-2300 million in Africa a year (Thresh *et al.,* 1997). Eggplant (S*olanum melongena*) is one of the cultivated favourite vegetables, which is grown both in tropical and sub-tropical regions (Schippers, 2000). Global annual production of eggplant worth $10 billon, which makes eggplant, the fifth most economically important solanaceous crop after potato, tomato, pepper and tobacco (FAO, 2014). Pests infecting eggplant crops are mainly insects and fungi. Very few viruses have been reported on eggplant especially emerging virus like begomoviruses (Schippers, 2000). Most important begomovirus infections were reported on food crops belonging to dicotyledonous families including *Euphorbiaceae* (Cassava), *Cucurbitaceae* (gourds, squash, watermelon and melon), *Malvaceae* (okra and cotton), *Fabaceae* (cowpea, mung bean, common bean, lima bean and soybean), *Convolvulaceae* (potato and sweet potato) and *Solanaceae* (tobacco, petunia, pepper and tomato) (Inoue-Nagata *et al.,* 2016). In Africa, the viruses reported infecting the eggplant are *Tomato mosaic virus* (Arogundade *et al.,* 2018), *Eggplant severe mottle virus* (Ladipo *et al.,* 1988) and the Potyviruses *Potato virus* X and *Potato virus* Y and the Tombusvirus *Eggplant mottle crinkle virus* (Chen *et al.,* 2001). Regarding begomoviruses, very few studies have been carried out on eggplant. In Asia, it has been reported the two begomoviruses *Tomato yellow leaf curl Kanchanaburi* begomovirus virus in Thailand (Green *et al.,* 2003) and *Tomato leaf curl New Dehli virus* in India (Pratap *et al.,* 2011). One of the most important steps in plant disease management is the identification of pathogens (Webster *et al.,* 2004). Therefore, this study is focused on the genome characterization of begomoviruses that infect eggplant in Cote d'Ivoire. *Enzyme linked-immunosorbant essay* (ELISA), *Polymerization chain reaction* (PCR) and *Rolling circle amplification* (RCA) are the common methods that are used in the detection of begomoviruses. The molecular tests PCR and the RCA show the highest sensitivity in the detection of begomoviruses than the antibodies essays like ELISA (Kushwaha *et al.,* 2010). In this study, the two molecular tests PCR first to detect begomoviruses and RCA to get full genomes components of begomoviruses were used.

MATERIAL AND METHODS

During this study 6 eggplant samples (11, 46, 47, 48, 49 and 50) among virus-like infected were collected. Sample 11 is from a farm in Ferkessoudougou in the northern area of Cote d'Ivoire while the other samples are from a farm in Sinfra in the southern area. Symptoms on the samples from farms are those cause by viruses. The symptoms on sample 11 were mainly yellow mosaic and leaves distortion while the ones on the 5 other samples were mainly leaf curling (Figure 1).

Figure 1: Symptoms on eggplant samples

A: Sample 11 from Ferkessedougou B: Sample 47 from Sinfra

Fresh sampled leaves were placed into paper envelopes and dried at 37° C for 72 hours in the laboratory in order to keep viral DNA from deterioration and to get more efficiency of DNA extraction and molecular tests (Thomson and Henry, 1993). The 6 samples were submitted to preliminary PCR screening for begomovirus detections. Total DNA was extracted using the adapted cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). The samples were pre-heated to 37°C for 12 hours and then ground to powder into test tubes containing bicycle balls. Total DNA extraction was done by the CTAB+beta-mercaptoethanol. The PCR reaction was carried out using the universal primers of begomoviruses P268/269 (Cluster4 F342/R1032 QIAGEN (TATMATCATTTCCACBCCVG-3 '/5'-GCATGAGTACATG CCATATAC-3') for direct PCR according to the program (95 ° C / 5 min, 95 ° C / 30 sec, 55 ° C / 40 sec, 72 ° C / 1 min, 75 ° C / 7 min) X 35 times. As the entire circular begomovirus DNA genome components was targeted, the rolling circle amplification (RCA) was performed on sample 11, which tested positive to PCR. The RCA process using *Phi29* DNA polymerase was used to amplify viral genome. The resulting amplicons were digested with *Bam*HI. After digestion, full-length DNA products were ligated into pGEM-3Zf vector (Promega) and cloned using *Escherichia coli* (Rector *et al.,* 2004). The detection was done in the *Laboratoire Mixte International* in Burkina Faso.

RESULTS AND DISCUSSION

PCR amplification and RCA clone sequencing : There was amplification within sample 11 by PCR but no amplification within the 5 other samples (figure 2). Otherwise, the study did not detect any begomovirus on eggplant samples with virus-like symptoms especially leaf curling infected suggesting the possibility of other virus. The clones obtained were sent for sequencing and results in contigs were assembled with Geneious 8.1 and consensus genome components of full-length DNA-A like of 2779 nt and DNA-B like of 2662 nt were obtained respectively. Preliminary sequence homology researches were done throughout the National Center of Biotechnology Information (NCBI) to identify potential relationship between the two nucleotide sequences and other known viruses in the database (Bao *et al.,*2004).

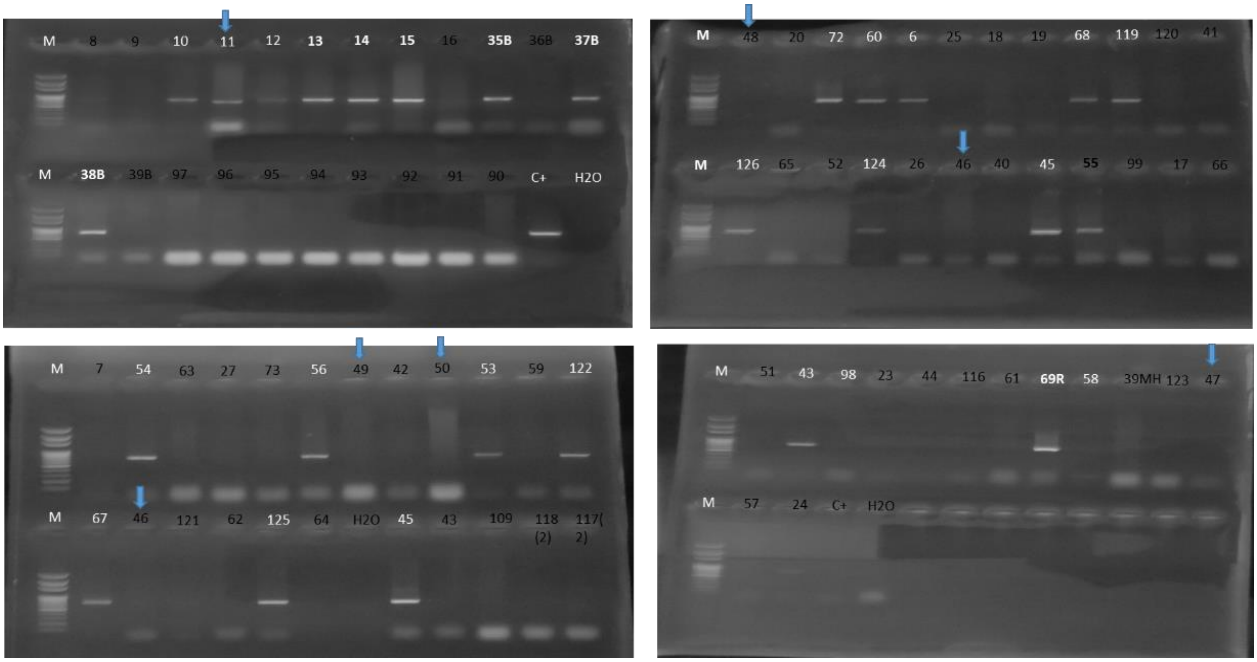

Figure 2: PCR amplification result on samples showing in different gel pictured, the presence of begomovirus sequences within the 6 samples of eggplant (11, 46, 47, 48, 49 and 50).

Sequence identification: A BLAST research with the sequence DNA A and a three colours matrix set from Sequence Demarcation Tool (SDTv1.2) with DNA A and other 18 begomoviruses indicated 98% nucleotide identity with *Pepper yellow vein Mali virus* (PepYVMLV) (Figure 3).

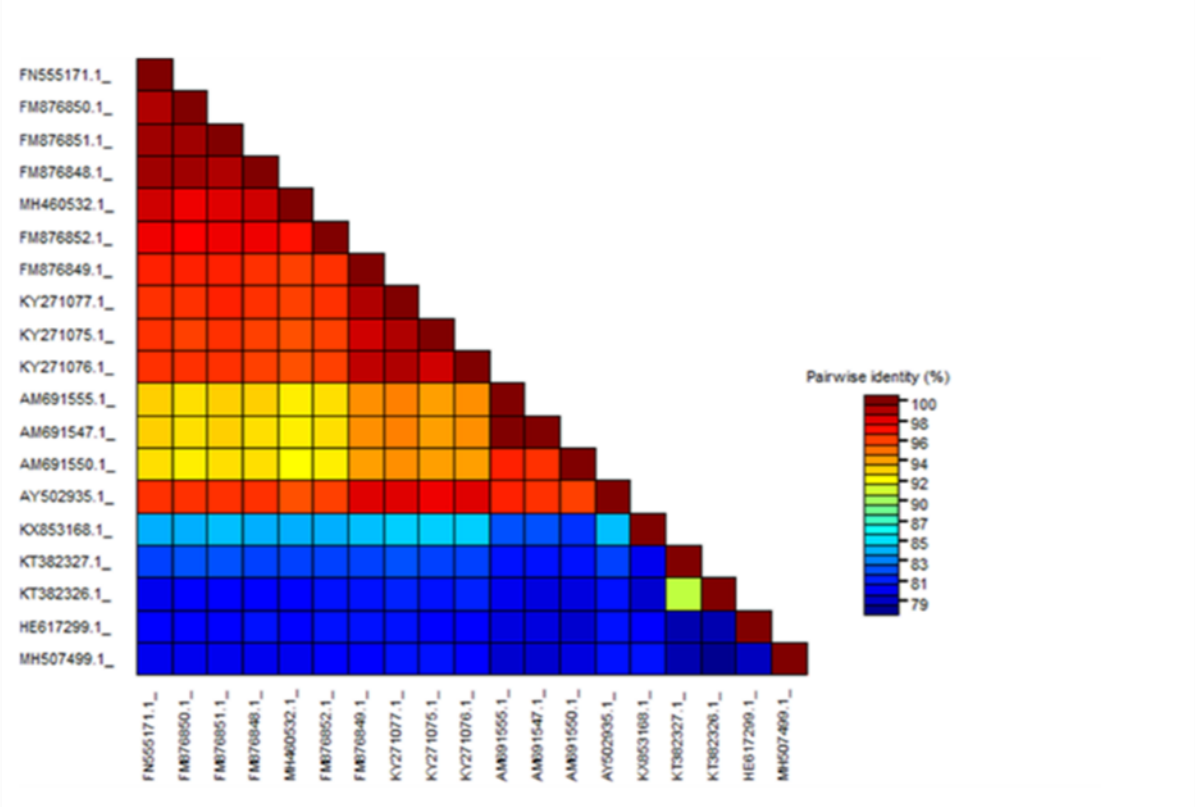

Figure 3: Three colours matrix of the sequence of DNA A [MH460532.1] and 18 other begomoviruses showing 98% identity with *Pepper yellow vein Mali virus* sequences. The scale bar shows the colour that correspond to the percentage of pairwise identity.

The BLAST searches indicated a nucleotides identity from 93 to 97% nucleotide identity between the sequence from DNA B-CI11 and the other PepYVMLV DNA B. identified on the sequence from DNA A, the six ORF of begomovirus DNA A component AV1 (virion-sense, 258aa, 299-1071), AV2 (virion sense, 116aa, 139-489), AC1 (complementary sense, 354aa, 1548-2612), AC2 (complementary sense, 135aa, 1217-1624), AC3 (complementary sense, 134aa, 1072-1476) and AC4 (complementary sense, 85aa, 2198-2455) which respectively encode capsid protein [CP], viral movement protein [MP], the replication-associated protein [Rep] and host range, transcriptional activator protein [TrAP], replication enhancer protein [REn] and viral accumulation. On the sequence from DNA B, we identified the two ORF of bipartite begomoviruses DNA B component BV1 (virion sense, 274aa, 369-1193) and BC1 (complementary sense, 306aa, 1257-2177) which encode respectively nuclear shuttle protein [NSP] and movement protein [MP] (Fondong, 2013). The components DNA A and DNA B of the same bipartite begomovirus species share about 200 nucleotides similarity in the common region (CR) in intergenic region (IR) CRA and CRB. The CRA and CRB from respectively the DNA A and DNA B are composed of 183 nucleotide sequence similarity. In order to know if DNA A and DNA B are from the same begomovirus species, we selected within the intergenic region, the common region (CR) sequences of DNA-A and DNA B, 15 other PepYVMLV and 4 closer bipartite begomoviruses. The SDT v2 software (Muhire *et al.,* 2014) was used to acutely calculate the pairwise nucleotide identity between the CR of DNA B-CI11 and other selected DNA sequences and we got as result a three colours matrix. The results from the matrix indicate scores of 92-95% and 89-90% pairwise nucleotide identity score between 89 and 95% with CR of the DNA B of PepYVMLV isolates (Table 1).

Table 1: Results of nucleotide pairwise identity between complete DNA A (MH460532.1) and the ones of PepYVMLV and other Begomoviruses; and the result of nucleotide pairwise identity between CR of DNA B (MH460533.2) and the ones of DNA A, other PepYVMLV and begomoviruses.

Accession number	Pairwise identity (%) of begomoviruses DNA A with DNA A (MH460532.1)	Pairwise identity (%) of begomoviruses DNA A CR with DNA B CR (MH460533.1)		
MH460532.1	100	92	PepYVMLV	Africa
FN555171.1	98	95		
FM876848.1	98	94		
FM876851.1	98	95		
FM876850.1	97	94		
FM876852.1	97	95		
FM876849.1	96	95		
KY271077.1	96	94		
KY271075.1	96	94		
KY271076.1	96	94		
AY502935.1	96	90		Asia
AM6915551	92	90		
AM691547.1	92	89		
AM691550.1	92	89		
KX853168.1	85	71	Other begomoviruses	
KT382327.1	82	74		
KT382326.1	80	74		
HE617299.1	80	74		
MH507499.1	80	74		

Otherwise, for other begomoviruses, there is score less than 75%. More interesting, the intergenic region CRA and CRB contains the characteristic conserved sequence of begomoviruses TAATATTAC and the TATA and GC boxes and the iteron TGGTAA. The corresponding iteron-related domain (IRD) in the N-Terminal region of the Rep was identified as MAPPKRFKIN. From the above explanation, DNA A-CI11 and DNA B-CI11 belong to the same species PepYVMLV (Briddon *et al.,* 2010; Moriones *et al.,* 2017). The DNA A-CI11 and DNA B-CI11 sequences have been deposited to GenBank which released them with the respective accession numbers MH460532.1 and MH460533.1 simultaneously in GenBank, European Nucleotide Archive and DNA Data Bank of Japan.

CONCLUSION and APPLICATION OF RESULTS

Few begomoviruses were reported on eggplant (*Solanum melongena*) worldwide. In Africa, no begomovirus was reported on eggplant (Brown *et al.,* 2015). Only the two begomoviruses *Tomato leaf curl New Dehli virus* and *Tomato yellow leaf curl Kanchanaburi virus* initially reported on tomato crops were also ultimately found on eggplant in Asia (Pratap *et al.,* 2011; Zhang *et al.,* 2018; Lukman *et al.,* 2019). In Africa, some begomoviruses were already reported on some Solanaceae such as pepper and tomato whereas neither on eggplant (Tiendrebeogo *et al.,* 2011; Séka *et al.,* 2017). A recent study carried out in Burkina Faso has reported begomovirus including PepYVML on pepper and tomato crops as well as on weeds but not on eggplant in the region (Ouattara *et al.,* 2019). It could be useful to infect eggplants in laboratory with the components DNA A and DNB of PepYVMLV in order to see the result interactions. The characterization of PepYVML from eggplant could help improve genetic plant management system in eggplant crops especially by setting resistant eggplant to PepYVMLV. From our knowledge, this is the first report on eggplant as a host of PepYVMLV.

CONFLICTS OF INTEREST

The authors have not declared any conflict of interest.

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